Validating the use of *Beggiatoa* sp. and opportunistic polychaete worm complex (OPC) as indicators of benthic habitat condition at finfish aquaculture sites in Newfoundland

D. Hamoutene, G. Mabrouk, L. Sheppard, C. MacSween, E. Coughlan, and C. Grant

Science Branch
Fisheries and Oceans Canada
P.O. Box 5667
St. John's NL Canada A1C 5X1

2013

Canadian Technical Report of Fisheries and Aquatic Sciences 3028

Fisheries and Oceans Canada | Pêches et Océans Canada

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts.*

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques.*

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Canadian Technical Report of
Fisheries and Aquatic Sciences 3028

2013

VALIDATING THE USE OF *BEGGIATOA* SP. AND OPPORTUNISTIC POLYCHAETE WORM COMPLEX (OPC) AS INDICATORS OF BENTHIC HABITAT CONDITION AT FINFISH AQUACULTURE SITES IN NEWFOUNDLAND

by

D. Hamoutene[1], G. Mabrouk[1], L. Sheppard[1], C. MacSween[1], E. Coughlan[2], and C. Grant[2]

[1]Fisheries and Oceans Canada
Science Branch
P.O. Box 5667
St. John's NL A1C 5X1

[2]Fisheries and Oceans Canada
Habitat Protection Division
P.O. Box 5667
St. John's NL A1C 5X1

© Her Majesty the Queen in Right of Canada, 2013.
Cat. No. Fs 97-6/0000E ISSN 0706-6457
Cat. No. Fs 97-6/0000E ISSN 1488-5379

Correct citation for this publication:

Hamoutene, D., Mabrouk, G., Sheppard, L., MacSween, C., Coughlan, E., Grant, C. 2013. Validating the use of *Beggiatoa* sp. and opportunistic polychaete worm complex (OPC) as indicators of benthic habitat condition at finfish aquaculture sites in Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 3028: v + 19 p.

TABLE OF CONTENTS

Page

ABSTRACT iv
RÉSUMÉ v
INTRODUCTION 1
MATERIALS AND METHODS 3
MONITORING REPORTS 3
SELECTION OF PARAMETERS AND CALCULATIONS 4
STATISTICAL ANALYSES 5
RESULTS 8
DATA DESCRIPTION 8
DECISION TREE ANALYSES 9
REPEATED MEASURES ANOVA 12
CORRELATIONS 12
DISCUSSION 13
REFERENCES 16

ABSTRACT

Hamoutene, D., Mabrouk, G., Sheppard, L., MacSween, C., Coughlan, E., Grant, C. 2012. Validating the use of *Beggiatoa* sp. and opportunistic polychaete worm complex (OPC) as indicators of benthic habitat condition at finfish aquaculture sites in Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 3028: v + 19 p.

In Newfoundland, approximately 90% of the finfish aquaculture sites have hard substrates often interspersed with patches of sand, gravel and/or mud. Biological and geological indicators have been developed to assess benthic conditions near aquaculture sites; however, most of these variables have not been fully assessed on hard and patchy substrates. In particular, conventional parameters, such as sulphides and redox potential used for soft sediments sites, have limitations in hard substrates because of the challenges associated with the difficulty of obtaining sediment grabs. The primary purpose of this study is to determine if two biological indicators, sulphur-oxidizing bacteria (*Beggiatoa sp.*) and opportunistic polychaete worm complexes (OPC) could be used to assess potential impacts of aquaculture operations. Habitat monitoring reports were used to obtain data collected over the finfish aquaculture production cycle (i.e., pre-production or baseline; in production; fallowed) at several sites in Newfoundland. The variability of *Beggiatoa* and OPC, as well as other indicators of interest, in conjunction with site conditions was analyzed using decision tree analyses (DTA), correlations and ANOVA. Statistical analyses' results show the validity of using *Beggiatoa* and OPC as well as other variables (e.g., flocculent matter, offgasing, redox and sulfides) as indicators of aquaculture impact on the benthic environment. The presence of *Beggiatoa* was confirmed as independent of substrate type or depth, and therefore a valid indicator of impact for both hard and soft bottom substrates. OPC presence was slightly influenced by depth but still higher at the end of production, with a significant decrease after fallow, showing a direct link with aquaculture operations. Results also reveal that *Beggiatoa* and offgasing are absent in reference sites, though OPC can be present (albeit rarely). Baseline values for redox potential and sulphides indicate that some sites are already post-oxic or slightly hypoxic even before the commencement of aquaculture activity. Another finding of this study is the importance of obtaining counts/abundance of fauna or flora identified in videos to ensure a better characterisation of the benthic populations.

RÉSUMÉ

Hamoutene, D., Mabrouk, G., Sheppard, L., MacSween, C., Coughlan, E., Grant, C. 2012. Validating the use of *Beggiatoa* sp. and opportunistic polychaete worm complex (OPC) as indicators of benthic habitat condition at finfish aquaculture sites in Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 3028: v + 19 p.

Dans la province de Terre Neuve, 90% des sites de pisciculture sont situés sur des fonds rocheux ou à composition variée (roche avec présence de sédiments fins, gravier, sable). Les indicateurs biologiques ou géochimiques utilisés traditionnellement pour évaluer l'impact de la pisciculture sur le benthos n'ont pas été validés sur ce type de fonds rocheux. Par exemple, la mesure des sulfures et du potentiel redox utilisés pour les fonds à sédiments fins ne peuvent être utilisés sur les fonds rocheux étant donné l'impossibilité d'obtenir des prélèvements grâce à une pelle mécanique. Le but essentiel de cette étude est de déterminer si deux indicateurs, une bactérie à métabolisme sulfurique (*Beggiatoa sp.*) et des groupements de vers de type polychète peuvent être utilisés comme indicateurs de changements benthiques dus à la pisciculture. Nous avons utilisé des données recueillies (rapports délivrés à la division de la gestion de l'habitat du poisson) à différentes étapes de l'existence de plusieurs opérations piscicoles et ce avant l'installation des cages, en fin de production, et après la période de jachère. Ces données ont été analysées en utilisant des arbres de décision, des corrélations et des analyses de variance. Nos résultats montrent la validité d'utiliser la présence de la bactérie *Beggiatoa*, des polychètes, des rejets de type floconneux, des gaz, du potentiels redox et des sulfures comme indicateurs d'un effet de la pisciculture et ce indépendamment du type de fond marin. La distribution des polychètes est quelque peu influencée par la profondeur néanmoins leur prolifération reste corrélée à la présence de la pisciculture. Nos résultats montrent également l'absence de *Beggiatoa* et gaz sur les sites avant l'installation des opérations aquacoles mais une légère présence de polychètes. De plus, nos résultats démontrent le fait que certains sites sont hypoxiques avant même l'installation des cages de pisciculture. Notre étude illustre également l'importance de la collecte des abondances des divers organismes benthiques pour pouvoir mieux caractériser les écosystèmes et ainsi évaluer plus clairement l'impact potentiel des rejets d'aquaculture.

1

INTRODUCTION

The impact of salmonid mariculture on hard substrates is not well understood. Although more than 120 biological and geochemical variables have been used to assess benthic condition near aquaculture sites (Kalantze and Karakassis 2006), none of these indicators have been fully assessed on hard substrates. Depending on site conditions, accumulation of organic waste (i.e., excess fish feed and faeces) on marine benthic habitats from finfish aquaculture operations has the potential to impact hard seabed biological communities. In the Newfoundland and Labrador (NL) region, the finfish aquaculture industry has expanded along the south coast of insular Newfoundland. This area has sheltered bays, coves, fjords, inlets, and freshwater lakes offering suitable areas for both cage and suspended gear culture. Currently, there are approximately 100 finfish aquaculture sites in Newfoundland, of which 30 to 40 are operational at any given time. Approximately 90% of the aquaculture sites have hard substrates (often interspersed with patchy substrates, such as sand) at depths greater than 30 m in accordance with DFO's siting criteria.

Two indicators, *Beggiatoa* sp. and opportunistic polychaete worm complexes (OPC), used on soft substrates may also have utility on hard substrates because they are conspicuous members of the benthic fauna and are known to occur near finfish sites on hard substrates. *Beggiatoa* may be a primary indicator as it occurs at the interface of oxic and anoxic conditions and is typically associated with elevated sulphide levels (Preisler et al. 2007). The proliferation of *Beggiatoa* mats over soft bottom sediments during periods of high organic input at finfish farms has been well documented (e.g., Crawford et al. 2001; Brooks and Mahnken 2003). A comprehensive review of the British Columbia Ministry of Environment (BCMOE) monitoring results collected at 21 active finfish farms under the Finfish Aquaculture Waste Control Regulation (FAWCR) of the *Environmental Management Act* has revealed that colourless, sulphur-oxidizing bacteria (*Beggiatoa* sp.) is also frequently observed under or adjacent to finfish farms sited on hard seabeds (Emmett et al. 2005, 2007, 2008). The second potential indicator, OPC, is frequently observed in areas with organic enrichment and reduced conditions (Brooks 2001). In particular, the proliferation of infaunal polychaetes within soft bottom sediments during periods of high organic farm input at finfish farms is well known (e.g., Brooks 2001). In addition, the review of the BCMOE video monitoring results collected around peak production at various sites in British Columbia (BC), revealed the occurrence of agglomerations of polychaetes in high densities on hard seabeds. In BC, the compliance standards at hard bottom sites already rely on the visual presence of *Beggiatoa* and OPC (http://www.pac.dfo-mpo.gc.ca/aquaculture/reporting-rapports/docs/benth/hardbottom-fonddur-eng.htm). Therefore, the use of these two indicators could be easily transferable to the aquaculture monitoring program over hard bottom substrates in NL. It is therefore important to confirm that the presence of both *Beggiatoa* and OPC on monitored sites in NL is indicative of the impact of aquaculture operations as it is in BC. Some of the more conventional parameters, such as sulphides

and redox potential used for soft sediments sites, have limitations in hard substrates because of the challenges associated with obtaining sediment grabs. Moreover, measurements of sediment redox potential, for example, have been found to be highly variable (e.g., Hargrave et al. 1993; Wildish et al. 1999). In North Carolina, Chanton et al. (1987) observed seasonal changes in dissolved sulphide flux over four years, with large summer increases in sediments which were not associated with aquaculture. Brooks (2001) recommended that redox potential continue to be evaluated as part of the salmon farm waste monitoring program in BC, but that it not be used as a trigger for biological monitoring. On the other hand, sediment-free sulphides are used as the primary physicochemical surrogate for regulating benthic effects at salmon farms in BC (Brooks 2001).

In this study, we will be using information gathered through baseline and industry monitoring to try and establish relationships between selected parameters and site characteristics. This will improve our understanding of the effects of aquaculture operations (extreme scenarios: no aquaculture, end of production, after fallow) on select parameters (*Beggiatoa* and OPC) and provide information as to whether they are valid indicators of habitat change. The current monitoring program in NL requires that video and grab samples be collected around the perimeter of the farm cages not more than two weeks before or two weeks after initiation of a fallow period of one year (Part 1) and four to eight weeks before a fallow period ends (Part 2) (DFO 2011). Monitoring is conducted by consultants and reports are sent to DFO's Habitat Protection Division (HPD) as part of the regulatory process. The objective of the monitoring program is to enable a farm operator to maximize yield at a fish farm site without causing a harmful alteration, disruption or destruction (HADD) of fish habitat, which is prohibited under Section 35 (2) of the *Fisheries Act*.

Through data extraction from the video description tables found in the baseline (reference conditions) and monitoring reports, we will explore how *Beggiatoa* and OPC, as well as other indicators such as offgasing, presence of flocculent matter and fauna and flora taxa, vary in conjunction with site conditions using decision tree analyses (DTA). DTA allows the formulation of relationships between one response (i.e., dependent) variable and several predictor (i.e., independent) variables by dividing a data set recursively into smaller, increasingly homogeneous portions. DTA allows the establishment of a hierarchy in the factors influencing the data (Hamoutene et al. 2009) while ensuring that the site scenario (i.e., baseline; in production; fallowed) and not the other conditions (i.e., depth and/or substrate type) have the strongest influence on the parameters assessed. Correlations between parameters will be explored, as will differences between values collected at the same sites at the different stages of production, as described above.

MATERIALS AND METHODS

MONITORING REPORTS

In addition to the baseline information collected on sites, the current monitoring program requires that video and grab samples be collected not more than two weeks before or two weeks after initiation of a fallow period. The baseline environmental survey was designed to enable both the proponents and the regulatory authorities to make informed decisions regarding site placement and site operation by providing knowledge of the seafloor composition prior to the implementation of aquaculture operations over a specific area of seafloor (i.e. prior to potential impact from an operating aquaculture farm). For baseline sampling, stations covering the site lease area are sampled over a 100 m grid. According to the Finfish Aquaculture – Farm Monitoring Report for Fish Habitat - Data Collection Instructions, established by Fisheries and Oceans Canada (NL Region), video recordings by drop/cable camera of the site bottom and grab samples showing the bottom conditions at cage edge are required. Video records of the seafloor are collected at cardinal points of each of the cages (four camera drops) as well as a panoramic continuous footage for at least one minute. When access to the four cardinal points at cage edge is difficult, consultants cover only two points at the cage limit. When sediment presence was indicated by video, triplicate samples were collected at each cage location, and redox and sulphide measured and recorded on each sample. It is important to note that in June 2011, HPD revised this protocol so that sampling occurs along transects rather than just at cage edge. The reports used for this study; however, only include data collected at cage edge. As stated earlier, the reports generated prior to fallowing are referred to as Part 1, and those established four to eight weeks before the end of a fallow period are referred to as Part 2. The reports used for this study had an average of 56.5 ± 30.4 stations (mean ± standard deviation) per site with video recordings for Baseline, as well as 53.5 ± 26.8 and 47.9 ± 31.7 stations for Part 1 and Part 2 reports, respectively. The data in the video description tables was summarized as described below, to obtain one unique observation per site. We analysed 26 Baseline reports, 14 Part 1 monitoring reports (14 different sites), and 18 Part 2 monitoring reports (18 sites). Contrary to expectation, Part 2 reports were not always completed 12 months after harvest, since fallow periods varied from five to 31 months. Only 10 sites had both the Baseline and Part 1 reports completed, while eight sites had both Part 1 and Part 2 reports completed. Unfortunately, not enough sites had both Baseline and Part 2 reports completed as some of the Baseline reports for the eight sites mentioned above had no usable data or were not available. Timing of sampling varied in the reports, which included data collected from January to December.

SELECTION OF PARAMETERS AND CALCULATIONS

Every video described in the tables listed in the Baseline/monitoring reports included the following information: GPS coordinates, depth, substrate type, a list of species/groups of fauna and flora present (no individual counts/abundances). The following groups were described in the tables for flora: eelgrass, green algae, brown algae, rockweed; and for fauna: echinoderms (feather star, brittle star, sea urchin, starfish), molluscs (mussels mostly), sponges, and anemones. Fish or other motile organisms are also reported when observed. The classification for fauna and flora was kept consistent throughout the process of data extraction to ensure the same level of detail was provided, so the mean numbers of groups would not be skewed by different methods of classifying the benthos. The following observations were also listed in the table, if present: flocculent matter, *Beggiatoa*, OPC, and offgasing. Similarly if a grab was obtained at the same stations and sulphides and redox potential were successfully measured, values were reported. A few examples of video descriptions are provided in Table 2; however, the GPS coordinates are not listed for confidentiality reasons.

As stated earlier, all video descriptions (one per station) were used to extract one unique observation/value per site, as follows:

- Flora and fauna (#): mean number of species/groups per station (in accordance to the level of details provided in the table), as no abundances were recorded by the consultants;
- Flocculent matter (%): data was summarized as a percentage of presence in all stations (number of stations with flocculent x 100 / total number of stations);
- *Beggiatoa* (%): data was summarized as a percentage of presence in all stations (number of stations with *Beggiatoa* x 100 / total number of stations);
- OPC (%): data was summarized as a percentage of presence in all stations (number of stations with OPC x 100 / total number of stations);
- Offgasing (%): data was summarized as a percentage of presence in all stations (number of stations with offgasing x 100 / total number of stations); and
- Substrate type: percentage of presence of the following classes of substrate was determined: bedrock, coarse (boulder, rubble), medium (cobble, gravel), and fine (sand, mud), as described in Table 1 (Wentworth 1922). For every substrate class, percentage was calculated as followed: number of stations with substrate class x 100 / total number of stations. As an example, a station can have a mixture of substrates present such as bedrock and sand; the presence of each substrate at that station would be recorded as 1 for bedrock and 1 for sand.

Similarly, data related to redox and sulfides measurements when available were used as follows:

- Mean redox potential (mV): mean of all values obtained (when applicable) at stations where grabs/measurements were possible;

- Mean sulfides (S^{2-}, µM): mean of all values obtained (when applicable) at stations where grabs/measurements were possible;

Table 1. Substrate classification based on particle size, adapted from Wentworth-Udden (Wentworth 1922).

Class	Description
Bedrock class	Bedrock (continuous solid bedrock)
Coarse class	Boulder (rocks > 250 mm); Rubble (rock ranging from 130-250 mm)
Medium class	Cobble (rocks ranging from 30-130 mm); Gravel (gravel size or coarser 2-30 mm)
Fine class	Sand (fine deposits ranging from 0.06-2 mm), mud (silt/clay < 0.6 mm)

A mean depth (in meters) for every site was also calculated as the mean value recorded at all stations as measured by the consultant (not based on chart datum).

STATISTICAL ANALYSES

As most parameters are expressed as percentages, data were arcsin square root transformed prior to statistical analyses, but this transformation showed no effect on results. Arcsin square root transformation is necessary when a sizeable number of the observed proportions are either relatively small ($P < 0.2$) or large ($0.8 < P < 1$); if most of the computed proportions lie between 0.2 and 0.7, it should have little impact on the results (Snedecor and Cochran 1980). DTA was used to explore trends in the parameters measured (dependent variables) in relation to site conditions (predictors). DTA allows the formulation of relationships between one response (i.e., dependent) variable and several predictor (i.e., independent) variables by dividing a data set recursively into smaller, increasingly homogeneous portions. Data used in this report were imbalanced, with more Baseline reports than Part 1 and Part 2 monitoring reports. Despite that, DTA allows us to reveal trends and conclude on influential factors. Exploratory analysis should be applied with no limiting assumptions about data distributions and independence of predictor variables (Breiman et al. 1984). The final result constitutes a division of the original data set into mutually exclusive and exhaustive sub-sets (e.g., Breiman et al. 1984; Quinlan et al. 1987; Biggs et al. 1991; Safavian and Landgrebe 1991; Hamoutene et al. 2008; Hamoutene et al. 2009). DTA was carried out using the procedure described by Breiman et al. (1984). At every level of the tree, stepwise splitting was performed by examining each of the predictor variables in turn, and selecting the predictor resulting in the smallest within-group sum-of-squares for a binary split. The splitting criterion was expressed as proportional reduction in error (PRE), with a minimum PRE of 0.05 required for a split to result for any given predictor/variable. The PRE constitutes the proportion of variance explained, whereby PRE is evaluated for each node as well as for the entire tree model (Breiman

et al. 1984). The procedure supports both continuous and categorical variables. The risk of overfitting was controlled by specifying a minimum number of cases, or stop size, for the creation of new nodes (Puestow et al. 2001). That is, if a given node contained fewer observations than the specified stop size, it was not further partitioned. A stop size of five was selected for all tree models. Dependent variables (i.e., the indicators) that may change with potential aquaculture impact include: flora and fauna, flocculent matter, redox potential, sulphides, *Beggiatoa*, OPC, and offgasing. Categorical predictor variables used in the analyses include 'report type': Baseline, Part 1, and Part 2. Continuous predictors are: 'depth' and substrate types (4 predictors) as divided in % 'bedrock', % 'coarse' , % 'medium', % 'fine'.

Comparisons between the indicators cited earlier were completed between Baseline and Part 1 reports (same sites), as well as Part 1 and Part 2, using repeated measures one-way ANOVAs. Correlations between potential indicators of impact, including mean number of flora and fauna groups, were also explored using the Pearson Product Moment correlation.

Table 2. Examples of video descriptions as found in the monitoring reports (these examples were taken from two different monitoring reports).

Cage number	Clock position	Coordinates		GPS accuracy	Depth (m)	Bottom type and condition	Description comments and observations	Date	Grab
		Lat.	Long.						
9P-18	N	Xxx	Xxx	0	63	sand, organic material, medium	mussels	10Sep-09	N
	W	Xxx	Xxx	3	64	bottom,	mussel shells, worms	10Sep-09	N
	S	Xxx	Xxx	4	66	flocculent on hard bottom, easily	mussel shells	10Sep-09	Y
	E	Xxx	Xxx	2	65	disturbed sand, hard packed sand/silt layer on cobble	mussel shells, flounder	10Sep-09	N
#1	W	Xxx	Xxx	0	20	hard bottom, organic material, cobble	shell debris , brittle stars, anemone, urchin	7May-10	Y
	N	Xxx	Xxx	3	22	hard bottom, organic material, cobble	shell debris, brittle stars, anemone, urchin	7May-10	N

RESULTS

DATA DESCRIPTION

The median values as well as maximum and minimum values of data extracted from the video analyses tables are summarized below (Table 3). Medians are presented because most of the data are not normally distributed. The aquaculture sites considered for this study show patchiness in substrate types and have a wide range of depths. Another noteworthy observation is the fact that *Beggiatoa* and offgasing are absent in Baseline reports. On the other hand, OPC was present (max=2.5%) on the benthos in the absence of aquaculture activity at one site only.

Table 3. Median, maximum, and minimum values extracted from the video description tables (and grab measurements for sulphide and redox potential) found in the Baseline and monitoring reports of all sites.

	Baseline (n=26)	Part 1 (n=14)	Part 2 (n=18)
Predictors			
Depth (m)	71.1 (15.0-194.2)	57.3 (37.4-101.4)	51.6 (23.4-96.5)
Bedrock (% presence)	0.0 (0.0-100.0)	71.6 (21.1-100.0)	25.8 (0.0-100.0)
Coarse (% presence)	20.0 (0.0-92.0)	7.0 (0.0-40.0)	20.5 (0.0-72.5)
Medium (% presence)	20.0 (0.0-71.4)	27.8 (0.0-90.0)	44.4 (7.2-90.0)
Fine (% presence)	53.7 (0.0-100.0)	62.0 (0.0-82.7)	64.6 (17.6-100.0)
Dependent variables			
Fauna (mean number of taxa)	0.04 (0.00-0.77)	0.15 (0.00-2.08)	0.45 (0.00-1.98)
Flora (mean number of taxa)	0.07 (0.00-0.77)	0.41 (0.00-1.00)	0.26 (0.00-1.53)
Beggiatoa (% presence)	0.0 (0.0-0.0)	62.4 (5.5-100.0)	40.4 (0.0-78.7)
OPC (% presence)	0.0 (0.0-2.5)	32.0 (3.1-100.0)	21.9 (0.0-65.0)
Redox potential (mean value, mV)	-72.0 (-155.3-417.3)	-325.6 (-407.7-161.3)	-290.0 (-411.9--197.0)
Sulphides (mean value, µM)	789.8 (440.9-823.5)	3588.6 (1037.6-7016.7)	1820.9 (214.5-4213.0)
Flocculent (% presence)	0.0 (0.0-3.9)	65.3 (17.5-100.0)	34.2 (0.0-72.0)
Offgasing (% presence)	0.0 (0.0-0.0)	20.4 (0.0-79.7)	10.6 (0.0-43.7)

DECISION TREE ANALYSES

All the site observations were combined in one unique data set to be used for decision tree analyses. Two examples of trees are provided below (Figures 1 and 2). Only the first two splits will be discussed (most trees had only two levels). The other trees were not added so as not to overcrowd the document. For *Beggiatoa* (Figure 1), Part 1 and Part 2 data are part of the same homogeneous group and have higher mean values than Baseline at the first split. Similarly, for OPC (Figure 2), the first split has Baseline values lower than Parts 1 and 2 grouped together, although the tree does not allow differentiation between Part 1 and Part 2 values.

The two main predictors, as well as the PRE for the entire tree for all dependent variables tested, are presented in Table 4. When the first predictor corresponds to the type of report, the first data subdivision is also presented in Table 4. The report type (site scenario) is the first predictor for most of the variables. For *Beggiatoa*, OPC, sulphides, as well as flocculent presence, the variability explained was above 60%. Aquaculture activity was the main influence on these variables, while depth and/or substrate type had less effect on the values recorded. For fauna, flora and offgasing, the PRE was below 60% with the substrate being the main predictor of the mean number of taxa for fauna, while the site aquaculture activity was the strongest influence on flora. Surprisingly, Baseline reports contained fewer numbers of taxa than Part 1.

Table 4. Main predictors and PRE values for decision trees generated for: fauna, flora, *Beggiatoa*, OPC, redox, sulphides, flocculent, and offgasing. Predictors include: substrate, depth, and type of reports.

Parameter	First Predictor and data subdivision	Second predictors and data subdivision	PRE
Fauna (n=57)	Substrate	Substrate	50.8%
Flora (n=57)	Type of reports (Baseline < Part 1 and 2)	Substrate	34.4%
Beggiatoa (n=57)	Type of reports (Baseline < Part 1 and 2)	Type of reports (Part 2 < Part 1)	75.4%
OPC (n=57)	Type of reports (Baseline < Part 1 and 2)	Depth	69.3%
Redox (n=32)	Type of reports (Baseline > Part 1 and 2)	Substrate	40.8%
Sulfides (n=32)	Type of reports (Part 1 > Baseline and Part 2)	Substrate	74.0%
Flocculent (n=57)	Type of reports (Baseline < Part 1 and 2)	Substrate	87.7%
Offgasing (n=56)	Type of reports (Part 1 > Baseline and Part 2)	Type of reports (Baseline < Part 2) and Substrate	58.8%

The number of observations n < 58 due to some missing data in one of the predictors and/or parameter.

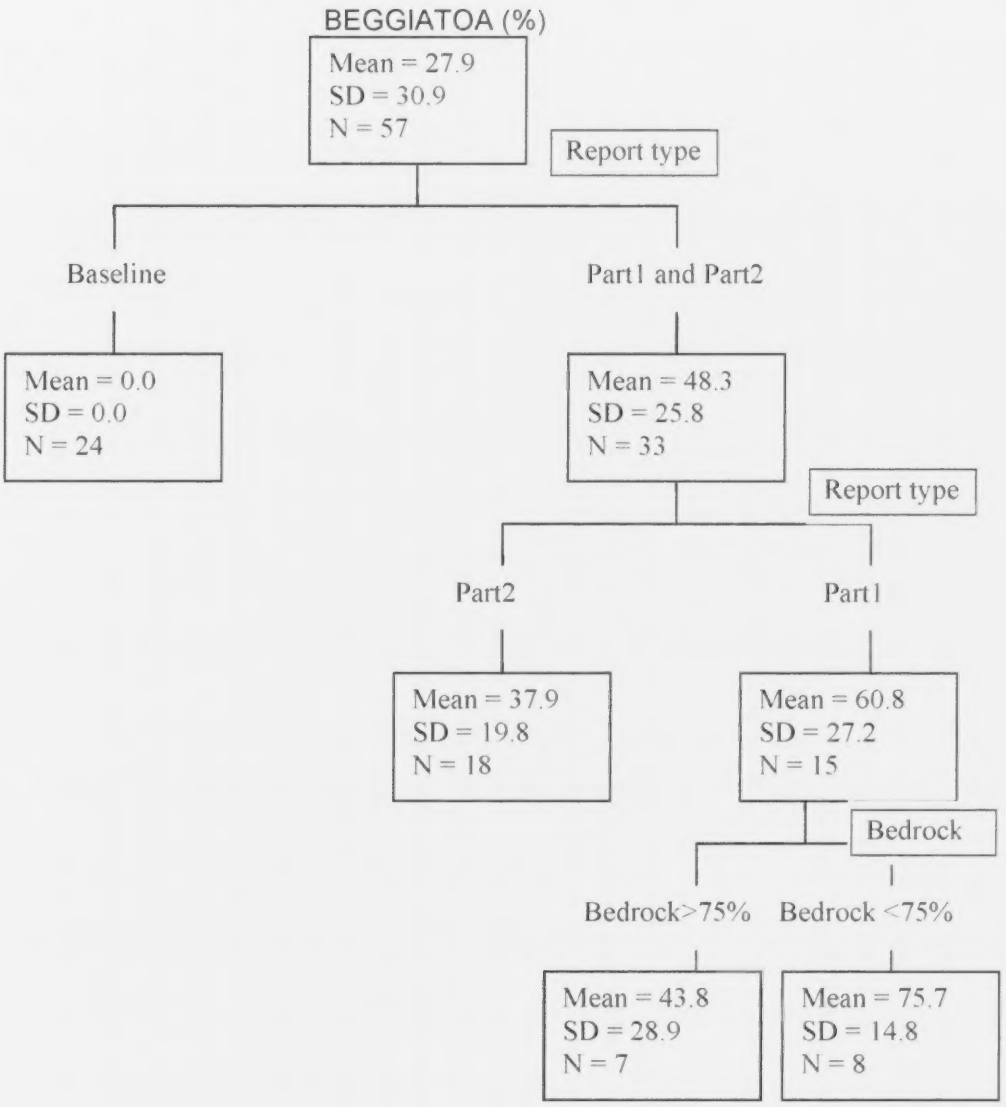

Figure 1. Decision tree generated with *Beggiatoa* data collected from 57 monitoring reports. Dependent variable is *Beggiatoa* % (values in the boxes) and predictors include: report type, depth (m) and bedrock, coarse, medium and fine substrate types (%). First two splits are determined by the report type (PRE of the tree is 75.4%). SD = standard deviation. N= number of reports.

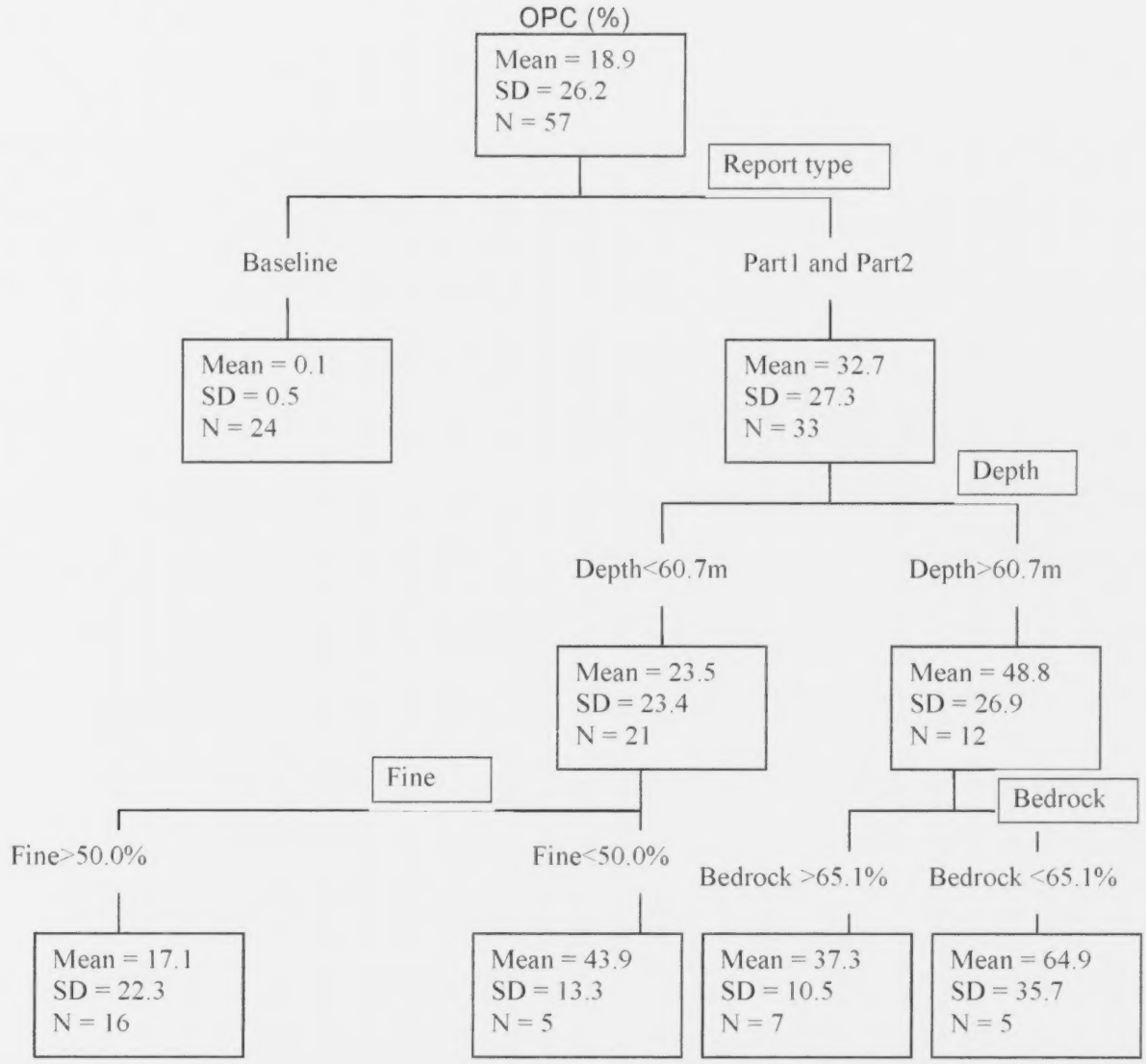

Figure 2. Decision tree generated with OPC data collected from 57 monitoring reports. Dependent variable is OPC % (values in the boxes) and predictors include: report type, depth (m) and bedrock, coarse, medium and fine substrate types (%). First splits are determined by the report type and second split is determined by depth (PRE of the tree is 69.3%). SD = standard deviation. N= number of reports.

REPEATED MEASURES ANOVA

For comparing Baseline and Part 1 reports, 10 sites were considered; for Part 1 and Part 2 comparisons, eight sites were used. There were too few sites with both Baseline and Part 2 monitoring completed to permit comparison between these two scenarios. As expected, differences were found in *Beggiatoa*, OPC, redox potential, sulphides, flocculent matter, and offgasing presence between Baseline and Part 1, with values being lower in the Baseline reports (Table 5). No differences were found in the mean number of flora and fauna present on the sites, though fewer flora groups were found in the Baseline reports when compared to Part 1. When comparing Part 1 and Part 2, *Beggiatoa* and OPC presence was lower in fallowed sites, though flocculent presence and offgasing were still prevalent even after fallow. OPC presence was recorded in 77.8% of the Part 2 reports and *Beggiatoa* in 94.4% of the cases.

Table 5. Comparisons between site status situations of the main indicators of change (fauna, flora, *Beggiatoa*, OPC, redox, sulphides, flocculent, and offgasing).

Parameter	Baseline-Part 1 comparison (n=10 sites)	Part 1- Part 2 comparison (n=8 sites)
Fauna	P = 0.232, no differences	P = 0.077, no differences
Flora	**P = 0.007, Baseline < Part 1**	P = 0.576, no differences
Beggiatoa	**P < 0.001, Baseline < Part 1**	**P = 0.004, Part 1 > Part 2**
OPC	**P < 0.001, Baseline < Part 1**	**P = 0.008, Part 1 > Part 2**
Redox	**P = 0.012, Baseline > Part 1**	Not enough data points
Sulphides	**P = 0.016, Baseline < Part 1**	Not enough data points
Flocculent	**P < 0.001, Baseline < Part 1**	P = 0.084, no differences
Offgasing	**P = 0.022, Baseline < Part 1**	P = 0.399, no differences

Significant correlations (P < 0.05) are highlighted in bold.

CORRELATIONS

Table 6 shows significant positive correlations between some known indicators of impact, such as *Beggiatoa*, OPC, sulphides, as well as flocculent presence and offgasing. No negative correlation was found between these indicators of change and the mean number of taxa, whether it be flora or fauna.

Table 6. Correlation coefficients between parameters as explored using Pearson Product Moment correlation (after Bonferroni adjustment, significance is at P < 0.002).

	Flora	Flocculent	Offgasing	Redox	Sulphides	*Beggiatoa*	OPC
Fauna	0.179	-0.073	-0.077	-0.031	-0.322	-0.016	0.047
Flora		0.219	0.102	-0.170	0.079	0.389	-0.005
Flocculent			**0.679**	- 0.462	**0.832**	**0.894**	**0.740**
Offgasing				-0.481	**0.625**	**0.538**	**0.416**
Redox					- 0.436	-0.430	-0.188
Sulphides						**0.747**	**0.632**
Beggiatoa							**0.606**

Significant correlations (P < 0.002 after Bonferroni adjustment) are highlighted in bold.

DISCUSSION

It is important to state that the data used for this study were collected at cage edge, therefore reflecting a worst case scenario situation and that the notion of zone of impact is not discussed as part of this report. Despite the fact that data were not balanced, DTA allowed us to reveal trends and conclude on influential factors. Exploratory analysis such as DTA was applied with no limiting assumptions about data distributions and independence of predictor variables (Breiman et al. 1984). In this study, the only assumptions made were that data from sites with different production levels and fallow period lengths (not all Part 2 reports were completed after one year of fallow as required) were considered as "equivalent" observations, and that the number of stations used to calculate these values could be different (i.e., different coverage). Sites with different conditions and sampling times (depth, substrate, production, etc.) were grouped in order to identify dominant trends, independent of specific site conditions, and to ensure that indicators such as *Beggiatoa* and OPC responded to aquaculture operations present throughout the year.

DTA results show the validity of using *Beggiatoa*, OPC, flocculent matter, and offgasing presence as well as redox and sulphides as indicators of aquaculture impact on the benthic environment. The decision trees demonstrated that the first predictor for all these parameters was 'type of reports' and that 'depth' and/or 'substrate types' had little or no influence on the results. *Beggiatoa* and offgasing were the only indicators that seemed "sensitive" enough to allow the differentiation between the three scenarios: baseline, end of production (Part 1), and end of fallow (Part 2). The one-way repeated measures ANOVAs between sites showed that, for a given site, offgasing values did not differ between Part 1 and Part 2 monitoring reports, suggesting that despite a fallow period this phenomenon is still observed. Other authors (e.g., Weston 1990; Holmer and

Kristensen 1992; Crawford et al. 2001) have found that white sulphur bacteria are visible in impacted sediments, showing that local benthic organic enrichment under fish cages can be detected by the presence of bacterial mats. The results presented in this report confirm that *Beggiatoa* presence is independent of substrate type or depth, and therefore a valid indicator of impact for both hard and soft bottom substrates. OPC presence is influenced by depth (second split). However, when comparing the same sites at the end of production and after fallow, OPC presence was higher in Part 1 reports, with a significant decrease after fallow, showing a direct link with aquaculture operations. The proliferation of infaunal polychaetes within soft bottom sediments during periods of high organic farm input at finfish farms has been well documented (e.g., Brooks 2001 and references therein). In contrast, there is very limited literature describing the physicochemical conditions of the seafloor at hard bottom sites (Hall-Spencer et al. 2006; Hall-Spencer and Bamber 2008). The review of the BCMOE video monitoring results collected around peak production at various sites throughout BC has revealed the occurrence of agglomerations of polychaetes in high densities on hard seabeds (Emmett et al. 2005, 2007, 2008).

No differences were found between site scenarios with respect to mean numbers of fauna groups, and a strong influence of substrate was found after the application of DTA. However, the absence of abundance/count data in the video tables precluded an accurate assessment of epibiota richness. Surprisingly, values for flora in Baseline reports were lower than in Part 1 and Part 2. This might be due to the fact, as cited above, that abundance was not evaluated, and that presence/coverage of many algae or plants would be recorded the same as an individual. Hard bottom substrates (i.e., rock, cobble and gravel) prevent using grabs or cores to collect samples, and water column sampling, underwater video or acoustic surveys are required to assess sediment and benthic community characteristics for monitoring purposes (DFO 2004, 2005). In the literature, most of the enrichment indices have been derived from observations in soft bottom areas of fine sand to mud sediments where sample collection was possible (Hargrave et al. 2008) rendering inferences from hard-bottom sites where only epibiota is evaluated difficult. In a study on aquaculture sites in Scotland, Henderson and Ross (1995) found that their results did not adequately establish cause and effect relationships between biological and sediment physicochemical endpoints, and suggested the need for a site-specific approach. After video assessment of environmental impacts of salmon farms, Crawford et al. (2001) showed that video data suggest an improvement in environmental conditions, while benthos samples implied a continuation of degraded conditions. This difference might be related to the fact that the video data represent the sediment surface, while the benthic community samples reflect conditions within the sediment (Crawford et al. 2001). Recovery after an organic enrichment event has been shown to occur more rapidly at the surface than within the sediments (Pearson and Rosenberg 1978). This was not observed in the data analyses described in this report, which is likely due to the fact that abundances were not recorded. Considering that video sampling is the primary

tool used for monitoring in the NL region, it would be useful to obtain counts/abundance of fauna or flora identified in videos to ensure a better characterisation of the benthic populations.

Notably, Table 3 reveals that *Beggiatoa* and offgasing are absent in reference sites, though OPC can be present (albeit rarely) on the benthos in areas where there is no aquaculture activity. Baseline values for redox potential and sulphides, as presented in Table 3, indicate that some sites would already be considered post-oxic or slightly hypoxic, as defined by Hargrave et al. (2008), even before the commencement of aquaculture activity. Therefore, if conditions are hypoxic before aquaculture impact, the potential for recovery processes would be impeded. Sulphides at reference locations away from fish cages were < 300 µM, while concentrations at farm sites varied from 150 to 5000 µM (S^{2-}) (Hargrave et al. 2008). The measurements collected in impacted sites (Part 1) correspond to the values described by Hargrave et al. (2008), though it is worth noting that Part 2 reports revealed persistent hypoxic conditions (median of 1820.9 µM) even after fallow, with OPC and *Beggiatoa* still present on the sites. Sediment chemical remediation following removal of salmon from culture cages has been defined as the return of organic and redox potentials to reference levels associated with a reduction of S^{2-} to < 960 µM (Brooks et al. 2003). Our data suggest that, despite a fallow period, sites still show hypoxic conditions and presence of indicators of organic enrichment such as *Beggiatoa*, OPC, flocculent matter, and offgasing. Variation in the duration of the fallow periods precluded any conclusions on trends.

Substrate description, as presented in Table 3 and evaluated by percent presence, illustrates a high degree of patchiness of the substrate types present on sites. Sites can consist of multiple types of substrates; for example 20% bedrock, 50% gravel (or medium substrate), and 30% mud (or fine substrates), thus rendering an overall classification of the substrate challenging. The presence of mud in areas with bedrock and overall hard substrates is associated with sedimentary "pockets". It is often hypothesized that the oxygen dynamics in these "pockets" may be similar to soft bottom sites, while oxygen levels in bare rock areas are likely rarely depleted, due to high current velocities, though no specific published literature is found on this topic (Emmett et al. 2005, 2007, 2008).

Some significant correlations are found between flocculent presence and offgasing, as well as *Beggiatoa*, OPC, and/or sulphides. No significant correlations were found between any of these indicators and redox potential values. In a report on the sediment physicochemical characteristics at seven salmon farms, Brooks (2001) found that sediment redox potential and free sulphides were negatively correlated, with Pearson correlation coefficients between -0.87 and -0.75. Similar results were also reported by Wildish et al. (1999). In this report, the absence of significant correlation with redox potential might be due to the low numbers of observations (no grabs), as well

as the inherent variability in measurements of sediment redox potential (e.g., Hargrave et al. 1993; Wildish et al. 1999).

Improvements and changes to the habitat monitoring protocols were implemented in June of 2011. In particular, the protocol has been changed from cage edge sampling to transect sampling (3 transects around the cage array). Moreover, a better quantification of OPC and *Beggiatoa* presence is completed through the evaluation of percent coverage using a frame of 50x50 cm or 25x25 cm reference. These changes are not discussed as part of this report, but the conclusions provided here allow us to confirm that performance-based indicators such as *Beggiatoa*, and OPC are biomarkers of aquaculture impact, independent of depth and substrate type. They can be used for regulatory purposes and correlate well with known indicators of aquaculture activities such as flocculent presence, offgasing and sulphides. Our results also suggest that benthic hypoxic conditions exist in many sites, so that any additional deposition may create anoxia, thus rendering probable recovery processes slower. This report highlights the importance of collecting counts/abundance of fauna and flora in order to better evaluate epibiota richness. Further focused research is required to identify specific compliance thresholds for *Beggiatoa* and OPC in relation to changes in benthic fauna and flora and to delineate a zone of impact.

REFERENCES

Biggs, D., De Ville, B., and Suen, E. 1991. A method of choosing multiway partitions for classification and decision trees. J. Appl. Stat. 18:49–62.

Breiman, L., Friedman, J.K., Olshen, R.A., and Stone, C.J. 1984. Classification and regression trees. Wadsworth International Group, Belmont. x + 358 pp.

Brooks, K.M. 2001. An evaluation of the relationship between salmon farm biomass, organic inputs to sediments, physiochemical changes associated with those inputs and the infaunal response –with emphasis on total sediment sulphides, total volatile solids and oxidation reduction potential as surrogate endpoints for biological monitoring. Technical Advisory Group, Ministry of Environment, Lands and Parks, Port Townsend, Washington. 172 pp. + appendices.

Brooks, K.M., and Mahnken, C.V.W. 2003. Interactions of Atlantic salmon in the Pacific Northwest II. Organic wastes. Fisheries Res. 60: 255-293.

Brooks, K.M., Stierns, A.R., Mahnken, C.V.M., and Blackburn, D.V. 2003. Chemical and biological remediation of the benthos near Atlantic salmon farms. Aquaculture 219: 355–377.

Chanton, J.P., Martens, C.S., and Goldhaber, M.B. 1987. Biogeochemical levelling in an organic rich coastal marine basin. 7. Sulfur mass balance, oxygen uptake and sulphide retention. Geochim. Cosmochim. Acta 51: 1187-1199.

Crawford, C.M., Mitchell, I.M., and Macleod, C.K.A. 2001. Video assessment of environmental impacts of salmon farms. ICES J. Mar. Sci. 58: 445-452.

DFO. 2004. A scientific review of the potential environmental effects of aquaculture in aquatic systems. Vol. III. Near-field organic enrichment from marine finfish aquaculture (Wildish, D.J., Dowd, M., Sutherland, T.F., Levings, C.D.). Environmental fate and effect of chemicals associated with Canadian freshwater aquaculture (Scott, R.J.). Can. Tech. Rep. Fish. Aquat. Sci. 2450, ix + 117 pp.

DFO. 2005. Assessment of finfish cage culture in the marine environment. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2005/034.

DFO. 2011. Finfish aquaculture- Farm monitoring report for fish habitat. Marine Environment and Habitat Management Division. Newfoundland and Labrador Region.

Emmett, B., Bornhold, B., and Burd, B. 2005. Evaluation of video and non-video, hard substrate seabed monitoring techniques. Phase 1 report. Prepared for BC aquaculture research and development committee, BC Innovation Council, 78pp.

Emmett, B., Thuringer, P., and Cook, S. 2007. Evaluation of hard substrate seabed monitoring techniques: development of video survey and data classification protocols. Phase 2 report. Prepared for BC aquaculture research and development committee, BC Innovation Council, 107pp.

Emmett, B., Thuringer, P., and Cook, S. 2008. Evaluation of hard substrate seabed monitoring techniques: development of compliance parameters from video survey data. Phase 3 report. Prepared for BC aquaculture research and development committee, BC Innovation Council, 27pp.

Hall-Spencer, J., and Bamber, R. 2008. Effects of salmon farming on benthic crustacean. Ciencias Marinas 33(4): 353-366.

Hall-Spencer, J., White, N., Gillespie, E., Gilham, K., and Foggo, A. 2006. Impact of fish farms on maerl beds in strongly tidal areas. Mar. Ecol. Prog. Ser. 326: 1-9.

Hamoutene, D., Puestow, T., Miller-Banoub, J., and Wareham, V. 2008. Main lipid classes in some species of deep-sea corals in the Newfoundland and Labrador region (Northwest Atlantic Ocean). Coral Reefs 27: 237-46.

Hamoutene, D., Lush, L., Drover, D., and Walsh, A. 2009. Investigation of the temporal effects of spawning season and maternal and paternal differences on egg quality in Atlantic cod *Gadus morhua* L. broodstock. Aquaculture Research 40: 1668-1679.

Hargrave, B.T., Duplisea, D.E., Pfeiffer, E., and Wildish, D.J. 1993. Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated with marine cultured Atlantic salmon. Mar. Ecol. Prog. Ser. 90: 249-257.

Hargrave, B.T., Holmer, M., and Newcombe, C.P. 2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. Mar. Poll. Bull. 56(5): 810-824.

Henderson, A.R., and Ross, D.J. 1995. Use of macrobenthic infaunal communities in the monitoring and control of the impact of marine cage fish farming. Aquat. Res. 26: 659-678.

Holmer, M., and Kristensen, E. 1992. Impact of marine fish cage farming on metabolism and sulfate reduction of underlying sediments. Mar. Ecol. Prog. Ser. 80: 191–201.

Kalantze, I., and Karakassis, I. 2006. Benthic impacts of fish farming: Meta-analysis of community and geochemical data. Mar. Poll. Bull. 52: 484-493.

Pearson, T.H. and Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanog. Mar. Biol. 16: 229-311.

Preisler, A., de Beer, D., Lichtschlag, A., Lavik, G., Boetius, A., and Barker Jorgensen, B. 2007. Biological and chemical sulphide oxidation in a *Beggiatoa* inhabited marine sediment. The ISME Journal, 1: 341-353.

Puestow, T.M., Simms, E.L., Simas, A., and Butler, K. 2001. Modeling of spawning habitat of Atlantic salmon using multispectral airborne imagery and digital ancillary data. Photogramm. Eng. Rem. S. 67: 309-318.

Quinlan, J.R., Compton, P.J., Horn, K.A., and Lazarus, L. 1987. Inductive knowledge acquisition: a case study. *In* Applications of expert systems. Edited by J.R.Quinlan. Tuning Institute Press, Sydney pp. 157–173.

Safavian, S.R., and Landgrebe, D. 1991. A survey of decision tree classifier methodology. IEEE Trans Syst Man Cybern 21:660–675.

Snedecor, M., and Cochran, C. 1980. Statistical methods, 7th ed. Iowa State University Press, Ames, IA, USA.

Wentworth, C. 1922. A scale of grade and class terms for clastic sediments. J. Geol. 30: 377-392.

Weston, D. 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. Mar. Ecol. Prog. Ser. 61: 233–244.

Wildish, D.J., Akagi, H.M., Hamilton, N., and Hargrave, B.T. 1999. A recommended method for monitoring sediments to detect organic enrichment from mariculture in the Bay of Fundy. Can. Tech. Rep. Fish. Aquat. Sci. 2286. iii + 31 pp.